Cellular Mechanisms of Acute Renal Failure in Rats

Versteilen, A.M.G.

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SUMMARY

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In this thesis we investigated several cellular mechanisms that contribute to the development of acute renal failure (ARF) and investigated the molecular mechanism of a Rho kinase inhibitor and a V_2-receptor agonist in respectively ischemia-reperfusion (I/R) and endotoxic shock induced ARF. In chapter 2 we reviewed mechanisms involved in I/R injury. Renal I/R is characterized by impaired endothelium-dependent vasodilatation and microvascular leukocyte accumulation, which contribute to the development of hypoperfusion, hypoxic injury and ultimately, acute kidney failure. Hypoperfusion of the kidney in ARF, despite appropriate support therapies, is a major cause of renal injury and acute tubular necrosis (ATN).

In chapter 3 we developed techniques to quantitatively analyze digital 3D-images and found evidence of microvascular injury indicated by fragmentation of the F-actin cytoskeleton of microvascular endothelial cells in the rat kidney following I/R. The methods of digital image analysis described in this study demonstrate that renal I/R is able to induce profound changes in the F-actin cytoskeletal structure of microvascular endothelial cells in situ. This might cause capillary hyperpermeability and endothelial cell detachment, resulting in impaired reperfusion of the renal microvasculature, aggravating renal parenchymal damage following I/R. This in turn diminishes glomerular filtration rate, leading to a rise in plasma creatinine, an event demonstrated here to correlate with endothelial F-actin cytoskeletal damage. Furthermore, collapse of the F-actin cytoskeleton is thought to represent a critical and sufficient event in the molecular pathways leading to apoptosis of endothelial cells. Finally, injury to the microvascular endothelium in the acute phase of ischemic ARF could result in the observed loss of capillaries in the outer medullary region, with concomitant functional defects and incomplete recovery – or even deterioration – of renal function on the long term.

In chapter and 4 we investigated the role of Rho kinase in the regulation of eNOS activity in renal arteries exposed to I/R. In this study, we have shown that inhibition of Rho kinase in renal I/R is able to restore renal perfusion by preventing decreased eNOS activity. Rho kinase

inhibition in renal I/R resulted in an increase in renal blood flow and NO-mediated ACh response in renal arteries, associated with an increase in p-eNOS and p-VASP in renal arterioles after I/R. In the next study, we investigated the cellular mechanism(s) underlying the previously reported Rho kinase-dependent regulation of eNOS activity in renal I/R. In particular, we investigated the possible relationship between Rho kinase-dependent regulation of eNOS distribution, alterations in Golgi complex morphology and microvascular leukocyte accumulation. This is the first study demonstrating that decreased eNOS activity following I/R may be associated with a Rho kinase-dependent intracellular redistribution of eNOS and a condensation of the Golgi complex in renal arteries. In addition, our data show that the capacity of Rho kinase to regulate eNOS activity is an important factor in leukocyte accumulation in the renal microcirculation following I/R.

Decreased eNOS activity in renal I/R might be the result of Rho kinase activation, mediated by COX-derived ROS. In Chapter 6 we investigated the role of COX and Rho kinase in the regulation of blood flow, the production of ROS in the arterial endothelium and vascular reactivity following renal I/R. We found that COX inhibition *in vivo* was not able to increase renal blood flow, or to decrease the concentration of ROS in the vascular endothelium following renal I/R, however, *in vitro* COX-inhibition improved endothelium-dependent vasodilatation. This in contrast to the results obtained in I/R animals treated with a Rho kinase inhibitor. In this group, blood flow was increased, ROS concentrations in the arterial endothelium were decreased and endothelium-dependent vasodilatation improved, without the COX inhibitor diclofenac. These results indicate that COX is not involved in Rho kinase activation, its downstream ROS production and the resultant decrease in blood flow and endothelium-dependent vasodilation following I/R in the rat kidney.

In chapter 7 we investigated the molecular mechanisms of an acute renal concentration defect following endotoxin infusion in rats. We found, in these animals as compared to controls, an increase in dilute diuresis, even after stopping saline infusion, suggestive of a renal concentration defect as commonly occurs during sepsis-induced ARF in man. In the presence of elevated vasopressin levels and a decreased inner medullary (IM) concentration gradient, a V_2-receptor signalling defect associated with

altered aquaporin 2 localization in renal collecting tubules, explains the observations. Strong stimulation with the selective V_2-receptor agonist desmopressin did not alter AQP2 localization, although it could raise the concentration gradient in the tip of the medullary papill to some extent and increase urinary concentration. In conclusion, our data suggest that during endotoxic shock in rats, AVP is released while the renal collecting duct cell fails to respond to the rise in AVP, associated with a fall and altered localization of AQP2. The selective V_2 receptor agonist desmopressin increased sodium reabsorption and IM osmolality, which stimulated water reabsorption resulting in a decreased urinary flow and increased urinary concentration, without changing AQP2 localization.

ARF due to hypoperfusion caused by alterations in vascular function (I/R) or a decrease in intravascular volume (endotoxic shock) is associated to high mortality rates in the intensive care units. In this thesis we have gained new insight in the molecular mechanism and therapeutic potential of a Rho kinase inhibitor and of a V_2-receptor agonist in respectively I/R and endotoxic ARF. The Rho kinase inhibitor was effectively in maintaining renal perfusion, vascular reactivity and preventing leukocyte accumulation following I/R due to regulation eNOS activity. In endotoxemia a V_2-receptor agonist increased the inner medullary osmolality, thereby stimulating water reabsorption contributing to the maintenance of intravascular volume. These drugs create the opportunity to limit the progression or speed up the recovery from ARF and hopefully in the near future the therapeutic potential of these drugs in humans will be further investigated.